

Supporting Information

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SI Text

TRAF6

5' ... UGCUCUA-GAAAGUUGAGUUCUCA-- (42nt) -- UCCUUGGAAAACUUAAGUUCUCA... TARGET
 ||||| ||| ||||| |||||
 3' UGGGUACCU--UAAGUCAAGAGU UGGGUACCUUAAG--UCAAGAGU miR146a

TRAK1

3' UUGGGUACCUUAAGUCAGAGU miR146a
 ||||| | | | | | | | |
 5' ...AAAUCC--GGAAGUCAAAGUUCUCAUGGUCAAGAAGUUCUCA... TARGET
 ||||| | | | | | | | |
 3' UUGGGUACCUUAAG--UCAAGAGU mir146a

PTC1

5' ...AUCAU--AAUACAGUUCUCA--(154nt)--UUGCCUUCAUGGUA--CAGUUCUCA... TARGET
 ||||| ||||| ||||||| ||||| ||||| |||||
 3' UGGGUACCUAAGUCAAGAGU UGGG-GUACCUAAGUCAAGAGU miR146a

Fig. S1. *IRAK1* and *TRAF6* are proven targets and *PTC1* is predicted to be a target of *miR-146* posttranscriptional repression. Shown is a sequence alignment of *miR-146a* and its target sites in the 3' UTRs of *TRAF6*, *IRAK1*, and *H4/PTC1*. The mutation constructs with 4-nt substitutions disrupting base-pairing within the “seed region” are also shown.

***IRAK1* (123nt)**

tgatgtttcacctggcagatccccaaatccgaagtcaaAGTTCTCATGGTCAGAAGTTCTCATGG
 TGCACGAGTCCTCAGCACTCTGCCGGCAGTGGGGTGGGGCCATGCCCGC
 GGGGGAGAGAAGGAGGTGGCCCTGCTGTTCTAGGCTCTGTGGGCataggcaggcag
 agtggAACCTGCCATGCCAGCATCTGGGGCAAGGAAGGCTGGCATCCAGTGAGGAGGCTGGCGATGTTGGAG

mutated: TTCT > aaga

***TRAF6* (78nt)**

aatcaactaccgccttgtcttagtgcctcgagaagagttattgtctagaAAGTTGAGTTCTCATTTTTTAA
 CCTGTTATAGATTCAGAGGATTGAACCATAATCCTGGAAAACTTAAGTTC
TCattcaccccagtttcctccagggttactaaggatattcaggatgagttaaac

mutated: TTCT > aaga

***PTC1* (192nt)**

tctatataaattttataatgttatgttaataatataatcataATACAGTTCTCAGATGCAGGGAAAGAA
 GTTTGGCATTAAATCATTGAGGCTTAGGTTTGATGTGATCAGACTGGGCC
 ATGTCAAACCCGAATTTCACCAACAGTCACTCACCCTCTGGTACATTGC
 CATTCCAAGGAATTCTGAGAGTAGGCAAACAAATTTCCTCATGGTACAG
TTCTCAGTtttcttataggagaatatggatatgtttataagaatctttatgagattatagattcaatgctgtggatagtgtct

mutated: TCTC > aaga

Fig. S2. *IRAK1* and *TRAF6* are proven targets and *PTC1* is predicted to be a target of miR-146 posttranscriptional repression. Shown are sequences of the 3' UTRs of *TRAF6*, *IRAK1*, and *PTC1* fused to the firefly luciferase gene in pGL3 Control Vector. The 4-nt substitutions disrupting base-pairing within the "seed region" (blue) are also shown.

Table S1. Expression of miR-146a by Northern blotting pictured in Fig. 1B

	tRNA	lac Z	Precursor		Mature			
			Raw	Normalized	Raw	Normalized		
pcDNA3	1,4461,223	275,705	206,669	52	1%	779,73	20	1%
C	15,695,290	218,462	6,796,294	1,982	53%	3,173,263	925	55%
G	17,023,091	286,827	18,363,509	3,761	100%	8,176,943	1,675	100%
C+G	16,162,365	306,113	12,079,482	2,442	65%	5,365,408	1,084	65%
C>G	17,151,508	263,602	25,302,517	5,596	149%	12,326,069	2,726	163%

The volume of the bands in the gel was quantified by using ImageQuant software (GE Healthcare). The expression of precursor and mature miR-146a was normalized to both lacZ (transfection efficiency) and tRNA-Glu (loading amount). The coefficient of variation is 12.2% for lacZ and 6.8% for tRNA. All normalized values were amplified by 10⁹ for the ease of reading. The normalized values are also shown as percentage of the expression of the G allele.

Table S2. Somatic mutations in tumor/normal tissue pairs

Sample name	Mutation (germ line > tumor)	No. of clones	Clones with mutated allele
Finn-PTC-009	GG>GC	52	15 (29%)
Finn-PTC-040	GG>GC	34	1 (3%)
Finn-PTC-041	GG>GC	50	2 (4%)
Finn-PTC-046	CC>GC	41	12 (29%)
Finn-PTC-109	CC>GC	51	13 (25%)
Finn-PTC-139	GG>GC	66	13 (20%)
Finn-PTC-145	GG>GC	36	15 (42%)
Finn-PTC-146	GG>GC	32	8 (25%)
USA-PTC-02	GG>GC	56	11 (20%)
USA-PTC-15	GG>GC	67	5 (7%)
USA-PTC-21	GG>GC	67	5 (7%)
USA-PTC-40	GG>GC	62	7 (11%)
USA-PTC-71	GG>GC	53	11 (21%)
USA-PTC-90	CC>GC	55	21 (38%)

Each tumor sample was TA cloned and individual PCR products genotyped. The frequency of clones exhibiting the mutation is shown.

Table S3. To exclude sample mislabeling seven microsatellite markers and the *BRAF* T1860A somatic mutation were tested in normal and tumor samples of all discordant tumor/normal pairs

	<i>BRAF</i> ^{V600E} in tumor	D3S3694	D3S1744	D3S1290	D5S2860	D10S1236	D10S1425	D16S752
Finn-PTC-009	Negative	136/142	140/154	210/216	205/205	124/134	170/178	N/A
Finn-PTC-040	Positive	134/134	136/150	208/218	185/213	128/128	178/178	108/116
Finn-PTC-041	Negative	144/144	150/150	220/220	185/205	122/122	166/174	104/104
Finn-PTC-046	Negative	134/142	140/146	210/218	205/209	N/A	178/178	108/116
Finn-PTC-109	Positive	134/142	150/158	216/216	201/205	124/124	166/178	116/120
Finn-PTC-139	Negative	134/134	150/158	212/212	205/205	124/134	178/178	108/116
Finn-PTC-145	Positive	136/142	150/154	216/218	185/205	128/130	166/182	112/116
Finn-PTC-146	Positive	190/190	136/146	210/216	205/209	126/128	166/178	108/116
USA-PTC-02	Positive	148/154	144/150	212/218	205/209	128/130	174/178	104/108
USA-PTC-15	Positive	142/150	144/150	212/212	201/205	126/128	174/178	112/116
USA-PTC-21	Positive	136/142	136/162	212/218	185/205	126/130	174/178	104/112
USA-PTC-40	Positive	142/142	136/150	212/222	205/205	126/136	178/178	108/112
USA-PTC-71	Positive	138/144	158/162	210/216	201/201	122/122	178/178	N/A
USA-PTC-90	Positive	138/150	150/150	208/218	201/209	122/122	170/174	N/A

Ten of 14 tumor samples were *BRAF*T1860A positive and all normal samples were negative. All samples within pairs were concordant as judged by every tested microsatellite marker.

Table S4. The expression of *miR-146a* and *miR-146b* by Taqman “stem-loop” real-time reverse transcription (RT)-PCR in 9 PTC tumor/normal pairs

		miR-146a			miR-146b			146a/b ratio	
		ΔC_T	$2^{-\Delta C_T}$	T/N	ΔC_T	$2^{-\Delta C_T}$	T/N		
1	PTC65	T	-4.00	16.01	2.13	-3.62	12.29	1.70	1.30
	PTC66	N	-2.91	7.50		-2.85	7.23		1.04
2	PTC73	T	-4.44	21.63	1.92	-11.50	2897.16	31.13	0.01
	PTC74	N	-3.49	11.27		-6.54	93.05		0.12
3	PTC75	T	-4.98	31.66	0.74	-9.42	685.64	16.42	0.05
	PTC76	N	-5.41	42.65		-5.38	41.74		1.02
4	PTC77	T	-4.70	26.02	2.75	-10.16	1144.22	102.26	0.02
	PTC78	N	-3.24	9.47		-3.48	11.19		0.85
5	PTC79	T	-3.45	10.95	1.81	-6.92	121.16	25.95	0.09
	PTC80	N	-2.60	6.06		-2.22	4.67		1.30
6	PTC83	T	-3.51	11.42	1.70	-9.84	916.05	92.85	0.01
	PTC84	N	-2.75	6.71		-3.30	9.87		0.68
7	PTC85	T	-4.00	15.96	1.63	-3.59	12.02	1.57	1.33
	PTC86	N	-3.29	9.76		-2.93	7.64		1.28
8	PTC89	T	-4.73	26.46	0.54	-7.91	241.27	5.22	0.11
	PTC90	N	-5.62	49.12		-5.53	46.22		1.06
9	PTC91	T	-4.49	22.43	1.43	-4.82	28.24	2.03	0.79
	PTC92	N	-3.97	15.70		-3.80	13.89		1.13

The cycle number at which the product level exceeded an arbitrarily chosen threshold (C_T) was determined for each target sequence, and the amount of each miR relative to U6 RNA was described by using the formula $2^{-\Delta C_T}$, where $\Delta C_T = C_{T(\text{miR})} - C_{T(\text{U6 RNA})}$.

Table S5. The expression of *miR-146a* and *mir-146b* by Taqman “stem-loop” real-time RT-PCR in 33 lymphoblastoid cell lines derived from healthy people

No.	Sample	<i>pre-miR-146a+60G>C</i>	miR-146a		miR-146b		146a/b ratio
			ΔC_T	$2^{-\Delta C_T}$	ΔC_T	$2^{-\Delta C_T}$	
1	KE111	GG	-6.57	94.95	-2.10	4.28	22.2
2	LA031	GG	-2.51	5.70	2.03	0.24	23.3
3	UU037	GG	-4.02	16.19	-0.39	1.31	12.3
4	UU038	GG	-6.61	97.80	-2.74	6.70	14.6
5	UU040	GG	-7.52	183.66	-2.83	7.11	25.8
6	KE113	GG	-3.75	13.49	0.28	0.83	16.3
7	UU008	GG	-4.07	16.77	0.03	0.98	17.2
8	VA011	GG	-6.98	125.92	-2.66	6.31	19.9
9	UU009	GG	-4.26	19.11	-0.17	1.12	17.0
10	KY106	GG	-5.16	35.80	-0.28	1.21	29.5
11	KY066	GG	-7.01	128.85	-3.35	10.19	12.7
12	VA010	GG	N/A	N/A	0.23	0.85	N/A
13	KY065	GG	-3.96	15.58	0.13	0.91	17.1
14	KU002	GG	-6.43	86.48	-1.56	2.94	29.4
15	OU015	GG	-7.15	142.40	-2.15	4.43	32.1
16	KE074	CG	-7.78	220.21	-2.02	4.04	54.5
17	PK017	CG	-5.72	52.60	-1.45	2.72	19.3
18	LA018	CG	-4.67	25.39	-0.17	1.12	22.6
19	UU036	CG	-5.66	50.73	-1.70	3.26	15.6
20	UU048	CG	-4.08	16.95	-0.35	1.27	13.3
21	KU059	CG	-8.50	362.94	-4.43	21.53	16.9
22	OU014	CG	-4.51	22.79	0.08	0.95	24.0
23	OU012	CG	-6.30	78.71	-2.12	4.34	18.1
24	HA105	CG	-8.09	271.75	-3.82	14.08	19.3
25	TU086	CG	-3.89	14.83	0.43	0.74	20.0
26	KY101	CG	-7.13	140.23	-2.63	6.19	22.6
27	OU020	CG	-6.61	97.86	-2.79	6.93	14.1
28	UU005	GC	-4.64	24.99	0.24	0.85	29.4
29	OU006	CG	-6.42	85.54	-2.75	6.74	12.7
30	VA013	CG	-3.51	11.42	0.94	0.52	21.9
31	UU007	CC	-3.13	8.73	1.21	0.43	20.2
32	UU061	CC	-4.78	27.38	-1.32	2.50	10.9
33	MI035	CC	-4.14	17.64	-0.04	1.03	17.2

The cycle number at which the product level exceeded an arbitrarily chosen threshold (C_T) was determined for each target sequence, and the amount of each miR relative to U6 RNA was described by using the formula $2^{-\Delta C_T}$, where $\Delta C_T = C_{T(\text{miR})} - C_{T(\text{U6 RNA})}$.

Table S6. The expression of *miR-146a* and *miR-146b* by Taqman “stem-loop” real-time RT-PCR in 11 samples derived from the unaffected part of the thyroid of PTC patients

No.	Sample	<i>pre-miR-146a+60G>C</i>	miR-146a		miR-146b		146a/b ratio
			ΔC_T	$2^{-\Delta C_T}$	ΔC_T	$2^{-\Delta C_T}$	
1	OSU-N34	GG	-5.95	61.99	-5.62	49.28	1.26
2	OSU-N40	GG	-5.36	41.16	-4.77	27.31	1.51
3	OSU-N13	GG	-5.40	42.33	-4.56	23.52	1.80
4	OSU-N17	GG	-5.60	48.54	-4.25	18.96	2.56
5	OSU-N09	GG	-5.13	35.10	-5.19	36.55	0.96
6	OSU-N36	GC	-5.81	56.25	-5.42	42.91	1.31
7	OSU-N38	GC	-6.31	79.33	-5.48	44.54	1.78
8	OSU-N39	GC	-5.28	38.96	-4.50	22.66	1.72
9	OSU-N15	GC	-8.87	468.73	-7.55	186.99	2.51
10	OSU-N20	GC	-5.96	62.35	-5.22	37.18	1.68
11	OSU-N20	GC	-2.83	7.11	-4.08	16.87	0.42

The cycle number at which the product level exceeded an arbitrarily chosen threshold (C_T) was determined for each target sequence, and the amount of each miR relative to U6 RNA was described by using the formula $2^{-\Delta C_T}$, where $\Delta C_T = C_{T(\text{miR})} - C_{T(\text{U6 RNA})}$.